RELATIONSHIP BETWEEN PLACENTOME LOCATION AND GENE EXPRESSION IN BOVINE PREGNANCY

A Thesis

by

ELIZABETH CHANTAL MACCONNELL

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Chair of Committee,	Michael Carey Satterfield
Committee Members,	Fuller Warren Bazer
	Andy D. Herring
Head of Department,	Graham Cliff Lamb

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ABSTRACT

We recently developed a novel, non-terminal surgical procedure to remove a single placentome from the pregnant ewe for gene expression and histological analyses. This technique allows evaluation of nutritional insults on placental development at more than one stage of gestation. However, the question remained of whether gene expression varied among placentomes based on location relative to the fetus. While this technique has not been developed in cattle, the similar shape of bovine and ovine placentomes led to this study in heifers. Pregnant heifers were maintained on forage during early gestation and later moved into pens with a Calan gate system (American Calan, Northwood, NH). On gestational day (GD)158, five heifers were assigned to receive a hay-based diet formulated to meet 100% of maintenance requirements, and five heifers were fed 70% of maintenance requirements until necropsy on GD270. At necropsy, a single representative placentome was selected from the antimesometrial side of: 1) the gravid horn central to the amnion, 2) over the allantois immediately adjacent to the amnion, 3) in the tip of the gravid horn, and finally 4) in the tip of the contralateral horn. Placentomes were removed, weighed, finely minced, snap frozen in liquid nitrogen and stored at –80^{o}C until samples were subjected to Real Time qPCR analyses. Mean weight of placentomes was greater ($P < 0.05$) for locations central to the amnion (74.3 ± 7.6g) and allantois (75.7 ± 7.6g) compared to locations within the tips of the ipsilateral and contralateral horns, respectively (25.9 ± 7.6g and 19.6 ± 7.6g). Gene expression for angiogenic factors (*FGF2*, *ODC1*, *VEGFA*, and *FLT1)*, nutrient transporters *(SLC7A1*,

and *SLC2A1*), and factors associated with hormone action *(ESR1, IGF1, IGFBP3, CSH1*, and *PAG1)* were unaffected ($P > 0.05$) by dietary treatment or location of the placentome. Results indicate that location of the placentome in relation to the fetus does not impact gene expression, enhancing the efficacy of non-terminal methodologies for sampling gene expression in placentomes.

DEDICATION

To Betty MacConnell.

The woman who instilled in me a passion for horses and for life. Rest in peace.

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CHAPTER I

INTRODUCTION

Environmental insults during pregnancy cause permanent structural, physiological, and metabolic changes as the fetus adapts to survive during critical stages of development (Godfrey and Barker, 2000; Godfrey, 2002). This phenomenon is termed fetal programming and can contribute to advantageous physiological adaptations in offspring as the fetus attempts to match its growth and metabolism to its predicted postnatal environment. However, programming can also be a considerable contributor to decreased postnatal efficiency when the fetus' postnatal environment does not match the fetus' predicted postnatal environment based on cues from the in utero environment. The beef cattle industry is especially susceptible to the mismatching of pre- and post-natal environments due to the lack of vertical integration. In the cow-calf sector; programming may occur frequently due to maternal nutrient restriction during gestation, however, producers often do not see negative effects as most calves are sold at weaning. Therefore, there is no incentive to increase input costs associated with increased dietary supplementation of dams. In turn, the stocker sector thrives on slightly thin calves to take advantage of compensatory growth, further perpetuating undernutrition of gestating cows. Therefore, the feedlot sector often sees the negative impacts of mismatched pre- and post-natal environments as cattle are rapidly finished on calorically high concentrate diets instead of nutrient poor diets which would match the prenatal environment of calves born to nutrient restricted dams.

The costs to the beef cattle industry due to decreased performance caused by mismatched pre- and post-natal environments have not been elucidated (Luther et. al., 2005; Wallace et. al., 2006). Often, these environmental insults during pregnancy result in deleterious effects on fetal development, such as intrauterine growth restriction (IUGR), which can be defined as a birth or fetal weight that falls into the lowest 10^{th} percentile for a given gestational age (McMillen et. al., 2001; Morrison, 2008). IUGR in livestock can be caused by many factors, including but not limited to, maternal undernutrition, overfeeding of growing dams, uterine infection, fetal chromosomal abnormalities, intra-uterine crowding, placentation malformations, and placental insufficiency (Schroder, 2003; McMillen et al., 2001, 2005; Reynolds et. al., 2009, 2010). Altered placental growth, vascular resistance, nutrient exchange, and hormonal action are all mechanisms contributing to placental insufficiency (Godfrey, 2002). Poor placental development beginning in early gestation can lead to poor substrate supply for adequate growth in late gestation causing IUGR (chronic substrate restriction; Harding et. al., 2000; Rees et. al., 1998; Morrison et al., 1997; Danielson et. al., 2005). Conversely, normal placental development may occur in early gestation, but a severe insult or multiple severe insults such as maternal nutrient restriction in mid-late gestation may still cause IUGR (acute substrate restriction or repeated acute substrate restriction).

Traditional approaches to the investigation of gestational insults on feto-placental development at the molecular level requires termination of the pregnancy and, therefore, study at a single point in time. Further, the terminal approach currently employed prevents retrospective assessment of correlations between postnatal phenotype and

placental gene expression in the same animal. A novel surgical technique to remove a single placentome during pregnancy in the sheep has been developed and allows collection of placental samples for evaluation while maintaining pregnancy. This technique could also allow for sampling of placental tissue at more than one time point during gestation from a single animal for stage of gestation comparisons without animal to animal variation. One potential concern with this approach is that the ruminant placenta is comprised of many placentomes throughout the uterus. Indeed, in both sheep and cattle there are 75 to 125 placentomes (Bazer et al., 2012; Furukawa et al., 2014; Dunlap et al., 2015). This led to the question of whether a single placentome would be molecularly representative of the entire population of placentomes from various regions within the gravid uterus. Answering this question would provide further information regarding the efficacy of the placentomectomy technique for analyses of stage of gestation effects on gene expression and/or advance knowledge of regional differences in functions of the ruminant placenta, specifically placentomes.

CHAPTER II

LITERATURE REVIEW

Fetal Programming Impacts

The current concept of fetal programming emerged from epidemiological studies in humans evaluating the relationship between maternal nutritional status and postnatal health of the resulting offspring (Hales et al., 1991; Osmond et al., 1993; Barker, 1998; Godfrey and Barker, 2000). The fetal origins hypothesis proposed that changes in fetal nutrition may cause structural, physiological, and metabolic adaptations by the fetus for short-term survival but would predispose the offspring for adult onset disease (Godfrey and Barker, 2000). Fetal programming in humans has been associated with an increased incidence of type 2 diabetes, coronary heart disease, high blood pressure, polycystic ovary syndrome, schizophrenia, insulin resistance, high cholesterol and obesity (Godfrey and Barker, 2000; Portha et al., 2011; Loche et al., 2018). One major consequence of fetal programming, low birth weight, was correlated with increased incidence of high blood pressure (Barker, 1998) and increased risk of death associated with coronary heart disease (Osmond et. al., 1993). Furthermore, the prevalence of type 2 diabetes and impaired glucose tolerance was observed in people who were small at birth, but obese as adults due to either insulin resistance (Hales et. al., 1991; Lithell et. al., 1996) or a reduced number of pancreatic β cells causing a lowered ability to produce insulin (Phillips et. al., 1994). Since the initial epidemiological findings, the rat has been a valuable research model due to its hemochorial placental type, monogastric digestive

system, short generation interval and the plethora of molecular biology techiniques validated in this species. Experimental studies in undernourished pregnant rats resulted in increased blood pressure in the offspring, similar to observations in humans (Woodall et. al., 1996; Langley-Evans et. al., 1996b). Furthermore, low protein diets for dams cause an increase in placental weight as well as fetal weight by GD20 however; fetal growth retardation from GD20 to birth in the rat causes offspring to have low birth weights compared to control offspring (Langley-Evans et. al., 1996b). Furthermore, low fetal weights have been reported at GD14 and GD18 due to low protein diets of dams (Gao et al., 2012). Offspring from protein restricted dams exhibit compensatory growth from birth to 4 weeks of age, but ultimately are smaller at maturity than offspring from control dams. They also exhibit impaired glucose homeostasis, insulin sensitivity, T killer cell function, and lymphocyte proliferation, which contributes to an overall shorter lifespan (Langley et. al., 1994a, 1994b; Hales et. al., 1996; Sayer et. al., 2001).

In addition to the rat, the sheep is a widely used model to evaluate effects of fetal programming on human health, but also retains the advantage over the rat of being applicable for the study of livestock performance (Bell, 2006; Reynolds et. al., 2010). The sheep lends itself to human studies with its relatively long gestation length compared to rodents, similar size to the human fetus allowing for fetal catheterization/sampling studies and are more developmentally similar to humans at birth than rodents (Bell, 2006). For example, the sheep model has been used to compare differing effects of maternal undernutrition during either early gestation or late gestation on the incidence of hypertension. Early gestational nutrient restriction has shown

conflicting results on the incidence of hypertension at 6 to 9 months of age (Gilbert et. al., 2005; Gopalakrishnan et. al., 2005), however, at 30 months of age, sheep born to undernourished ewes were clearly hypertensive (Gopalakrishnan et. al., 2004). Furthermore, as early as postnatal day 21, twin lambs with a 30% reduction in birth weight compared to singleton lambs possessed increased concentrations of sodium in plasma as well as increased diastolic, systolic, and mean arterial blood pressure (Ross et al., 2005). Maternal nutrient restriction during late gestation did not cause hypertension in offspring at 30 months of age (Oliver et al., 2002).

Conversely, research in fetal programming has branched out to evaluate the effects on livestock as it relates to animal health and production characteristics. In livestock, many stressors can cause programming adaptations including maternal over- and under-nutrition, uterine crowding, heat or environmental stress, or pregnancy in growing dams. However, the main environmental factor proven to cause fetal programming adaptations in food animals is under-nutrition due to multiple production scenarios (Reynolds et. al., 2010). First, females are often peripubertal and still growing during their first pregnancy which results in competition between maternal and fetal needs for nutrients. Second, regardless of selection for increased mature maternal size, selection to increase reproductive efficiency in species that twin (sheep or goats) or have litters (pigs) has increased numbers of fetuses, leading to a decrease in fetal growth due to intra-uterine crowding. Third, selection for increased milk production has created competition for nutritional resources required for fetal and placental growth and those required for lactation. Fourth, seasonal changes in pasture conditions may lead to a lack

of nutrient value in forage consumed by the dam during critical stages of gestation (Reynolds et. al., 2010). Poor nutrient availability due to variability among pastures in quality of forage is the most relevant potential cause of nutrient restriction to cow-calf operations. Due to the frequency of poor nutrient availability in production settings, a growing number of studies have evaluated the effects of nutrient restriction during gestation on the offspring's long-term health, reproductive performance, and carcass characteristics. With the more economical cost and shortened gestation length when compared to cattle, sheep have been widely used to evaluate the impacts of fetal programming in livestock. However, more research on fetal programming in cattle is needed to evaluate the association between poor maternal nutrition and health, production, and economic consequences in the cattle industry.

Organogenesis

Fetal organogenesis is altered by insults like nutrient restriction during early gestation. During early embryonic development, three germ layers form. The innermost, endoderm, will differentiate into the digestive, respiratory, and urinary tracts as well as the endocrine glands (Lemley et. al., 2015). The intermediate layer of cells, the mesoderm, will differentiate into cardiac, smooth, and skeletal muscle as well as the circulatory system and reproductive tract (Lemley et. al., 2015). The outermost layer of cells, ectoderm, will differentiate into the nervous and integumentary systems (Lemley et. al., 2015). Organogenesis and the majority of cellular differentiation occurs in early gestation making organ function susceptible to environmental insults which may impact the lifelong health of the offspring. The bovine heartbeat is apparent at GD day 21

(Lemley et. al., 2015). Limb development begins from GD 25 to 30 however; limbs begin to elongate at GD50 (Lemley et. al., 2015). Differentiation of the germ layers into organs such as the rumen, reticulum, omasum, pancreas, liver, lungs, adrenals, thyroids, muscle and kidneys begins in the bovine fetus from GD40 to 50 (Lemley et. al., 2015). In an effort by the fetus to survive, priority of nutrient partitioning goes to critical organs like the heart and brain, if exposed to restricted conditions (Lemley et. al., 2015). The heart is most susceptible to gene dysregulation, such as downregulation of VEGF, during organogenesis while the kidney is least affected by gene dysregulation during organogenesis in cloned calves that died within two days of birth (Li et al., 2005). Maternal nutrient restriction from GD30 to 125 decreased absolute weight of the brain in fetuses at day 125; however when expressed as a proportion of total fetal weight, weight of the brain was actually greater than that for control-fed calves. Furthermore, fetal heart, lung, and liver weights were decreased in nutrient restricted calves compared to control-fed calves (Long et. al., 2009). When cows are re-alimented from a nutrient restricted diet at GD125, organ and fetal weights are unaffected by treatment at GD245 (Long et. al., 2009).

Compensatory growth occurs if dams are given above maintenance nutrition during mid and late gestation as fetal growth rate is greatest on GD232 in cattle (Prior and Laster, 1979). However, there may be lifelong consequences due to these fetal adaptations. Steers exposed to nutrient restriction in utero had decreased lung and trachea weights at slaughter (Long et. al., 2010). Negative effects on the respiratory system caused by nutrition in utero could potentially have an effect on the incidence of

bovine respiratory disease (BRD) in finishing cattle. Decreasing contributing factors to the incidence of BRD has the potential to greatly affect the profitability of the feedlot sector as BRD is the largest cause of morbidity and mortality in feedlot cattle (Taylor et al., 2010). Not only is organ function susceptible, but programming of adipocyte development can cause major impacts on survival and carcass performance of cattle. White adipocytes are first detectable around GD80. Brown adipocytes, which function to generate heat at the time of birth to support thermoregulation of the neonate, first appear at GD190 in cattle (Lemley et. al., 2015). Even the gonads are vulnerable to programming as testicular development begins around GD45 while ovarian development begins around GD50 to 60 (Lemley et. al., 2015; McGowan et al., 2018). Furthermore, primordial germ cells are located close to the undifferentiated fetal gonad by GD23 to 25 and then populate the gonadal ridge from GD27 to 40, making developmental events in early gestation susceptible to programming (Lemley et. al., 2015). For example, maternal nutrient restriction is associated with a 60% reduction in antral follicle counts (AFC) in heifers born to dams fed 60% of their energy requirements (Mossa et al., 2013). Maternal undernutrition during the first 110 days of gestation in sheep also reduces the number of follicles that develop beyond the primordial stage even if the restriction is limited to 1 or 2 months during this period of gestation (Rae et. al., 2001). Conversely, *Bos indicus* cross heifers from dams overfed during gestation had a reduction in density of primordial and primary follicles, but AFC was unaffected by nutritional treatment (Sullivan et. al., 2009). Those results suggest that deficits or

excesses in maternal nutrition during gestation have a negative impact on fetal organogenesis that is detrimental to health and performance of offspring.

As in the early epidemiological studies of livestock, birth weight is often used as a proxy measurement for the quality of the intrauterine environment within which the fetus developed. Nonetheless, the effect of maternal nutrient restriction on birth weight of calves has yielded variable results (Table 1). Some studies found maternal nutrient restriction during mid gestation to decrease fetal weights at GD125 and at birth (Long et. al., 2009; Micke et. al., 2010). However, many studies found no difference in fetal or birth weights of calves from nutrient restricted dams (Martin et. al., 1997; Long et. al., 2009; Underwood et. al., 2010; Long et. al., 2010; Summers et. al., 2015; Paradis et. al., 2017). Discrepancies between fetal or birth weights in these studies may be attributed to age differences with heifers having higher nutritional demands than mature cows (Martin et al., 1997; Micke et al., 2010; Long et al., 2010; Summers et al., 2015) as well as differences in duration and severity of nutrient restriction, and the periods

Birth Weights

Table 1: Summary of studies evaluating effects of maternal nutrient restriction (NR) compared to control (C) diet on offspring birth weight.

Reference	Dam Breed Type	n	Period of Restriction	Severity of Restriction	Day of Observation	Observation
Long et. al., 2009	Multiparous Angus X Gelbvieh cows	NR=10 C=10	GD30 to GD125	68.1% of NEm; 86.7% of MP	GD125	6 NR Non-IUGR; 4 NR IUGR
Long et. al., 2009	Multiparous Angus X Gelbvieh cows	NR=5 C=5	GD30 to GD125	68.1% of NEm; 86.7% of MP	GD245	No difference in fetal weights due to treatment
Underwood et. al., 2010	Crossbred beef cows	NR=12 IP=14	GD120-150 to GD180-210	IP (6-11.1% CP); NR (5.4-6.5% CP)	Birth	No difference in birth weights due to treatment
Paradis et. al., 2017	Multiparous Simmental X Angus cows	85%=12 140%=12	GD147 ± 15 to GD247 ± 10	140% ME; 85% ME	GD247 ± 10	No difference in fetal weights due to treatment
Long et. al., 2010	Angus X Hereford heifers	55%=10 100%=10	GD32 to GD82	55% of NRC; 100% of NRC	Birth	No difference in birth weights due to treatment
Summers et. al., 2015	Angus based crossbred heifers	YR 1: 38 YR 2: 40 YR 3: 36	GD167 to GD251	non-supplemented; (High) 59% RUP; (Low) 34% RUP	Birth	No difference in birth weights due to treatment
Micke et. al., 2010	1/2 Senepol 1/4 Brahman 1/4 Charolais **or** 1/2 Senepol 1/4 Brahman 1/8 Charolais 1/8 Red Angus heifers	HH: 16 HL:19 LH:17 LL:19	GD0 to GD180	(High): 76Mj of ME and 1.4kg CP (Low): 62Mj of ME and 0.4kg CP	Birth	Low diet during second trimester decreased birth weights
Martin et. al., 1997	Angus heifers	8 per treatment	d99 to birth	10.4% Protein; 6.8% Protein	Birth	No difference in birth weights due to treatment

Table 1 Continued

IP = Improved pasture
NR = Native range
ME = Metabolizable energy
YR = Year
HH = High plane of nutrition during early and mid- gestation
HL = High plane of nutrition during early gestation and low plane of nutrition during mid- gestation
LH = Low plane of nutrition during early gestation and high plane of nutrition during mid- gestationLL = Low plane of nutrition during early and mid-gestation

of gestation during which nutrient restriction occurred. Fetal growth rate is dynamic and follows an exponential trajectory until very late in gestation (Strickland, 1978; Lemley et. al., 2015). From GD70 to 100, the bovine fetus grows at a rate of 10g/day (Lemley et. al., 2015). In contrast, from GD200 to 250, fetal growth increases to 200 to 300g/day and then decreases in late gestation to 100g/day (Lemley et. al., 2015). One study evaluated calf performance at GD125 and GD245 when cows were restricted from GD30 to GD125 and subsequently realimented until GD245. At GD125, fetal weights were reduced in nutrient restricted dams, however, no reduction in fetal weight occurred at GD245, likely due to compensatory fetal growth during the realimentation period (Long et. al., 2009). Nutritional restriction models also vary in regards to the type of nutrient deficiency. While some models utilize a global nutritional restriction (Long et al., 2010), other models specifically target protein (Martin et al., 1997; Underwood et al., 2010; Summers et al., 2015) or energy restriction (Long et al., 2009; Micke et al., 2010; Paradis et al., 2017). For example, metabolizable energy and crude protein restriction in heifers during the second trimester of pregnancy reduces fetal growth and birth weight (Micke et. al., 2010) but protein restriction alone during the third trimester in heifers did not decrease birth weight at calving (Martin et. al., 1997). Additional studies are needed to characterize differences in birth weights of offspring due to factors such as dam parity or breed type, as well as timing, duration, and severity of restriction.

Muscle and Fat Development and carcass performance

Fetal skeletal muscle development has a lower priority for nutrients than organs like the brain, heart, and liver; therefore, it is more susceptible to decreases in nutrient partitioning during maternal nutrient restriction (Zhu et. al., 2006). Myocytes, adipocytes, and fibroblasts originate from the same stem cell pool and make up the structure of the skeletal muscle (Du et. al., 2010). Muscle development begins in the embryonic stage with primary myofibers developing from the mesodermal cell layer of the embryo (Du et. al., 2010). Secondary myofibers which develop during the fetal stage of gestation account for the majority of the myofibers (Ward et. al., 1991). The ratio of secondary to primary myofibers is lower in muscles with predominantly slow oxidative fibers (Ward et. al., 1991). Secondary myofiber development overlaps partially with intramuscular adipocyte and fibroblast development (Du et. al., 2010). Postnatal muscle growth is mainly due to increases in muscle fiber size, instead of increases in muscle fiber numbers (Stickland, 1978; Karunaratne et. al., 2005; Du et. al., 2010). In mature muscle fibers, satellite cells are located between the basal lamina and the sarcolemma and function to fuse with muscle fibers to allow for muscle growth in postnatal life (Kuang et. al., 2007). A small number of satellite cells are multipotent and can differentiate into fibroblasts or adipocytes instead of myogenic cells (Aguiari et. al., 2008; Kuang et. al., 2008; Yablonka-Reuveni et. al., 2008). Carcass value in cattle is determined by yield grade and quality grade. Carcass yield grade is determined by the amount of lean muscle compared to intermuscular and subcutaneous fat with higher value given to carcasses with more lean muscle. Development of skeletal muscle during

gestation is crucial for livestock production because there is no substantial increase in muscle fiber numbers after GD240 (Paradis et. al., 2017). Therefore, fetal programming that decreases the number of muscle fibers decreases overall muscle mass, negatively affecting animal performance (Du et. al., 2010). During bovine fetal development, primary myofibers form within the first 2 months of gestation (Russell and Oteruelo, 1981). However, with the small number of primary myofibers in comparison to secondary myofibers, an insult like nutrient restriction during early gestation does not have significant effects on muscle development (Du et. al., 2010). However, there are conflicting results as steers exposed to early gestational nutrient restriction have larger, but fewer muscle fibers (Long et. al., 2010). In addition, secondary myofibers develop from 2 to 7 or 8 months of gestation and constitute the larger majority of myofibers (Russell and Oteruelo, 1981). Nutrient restriction during these later months of fetal development decreases development of secondary myofiber numbers which has life-long negative impacts on the offspring (Zhu et. al., 2006). Early- to mid- gestational nutrient restriction causes decreased muscle fiber numbers and muscle mass in skeletal muscle of the offspring in sheep (Zhu et. al., 2006; Quigley et. al., 2005), pig (Dwyer et. al., 1994), and guinea pig (Ward and Strickland, 1991). Bovine skeletal muscle matures around GD210 and continues to increase in muscle fiber diameter, but not muscle fiber number (Greenwood et. al., 1999). Nutritional effects on secondary myofibers may be controlled by innervation (Ward and Strickland, 1991). In addition to muscle fiber numbers being altered by fetal programming, muscle fiber type is an important factor to evaluate. Type I myofibers have a lower growth efficiency and greater protein turnover rates while type

II myofibers possess greater growth efficiency and reduced catabolic rates (Du et. al., 2010). Lambs from nutrient restricted dams possessed a higher percentage of type II myofibers compared to lambs from control fed dams (Zhu et. al., 2006). This represents the fetus's ability to adapt to a more efficient phenotype while still in a state of developmentally plasticity.

Carcass quality is determined by the amount of intramuscular fat (marbling) with more valuable carcasses having a higher incidence of marbling. Intramuscular fat is developed during gestation and can be affected by fetal programming mechanisms, leading to decreased carcass quality and, therefore, decreased value (Tong et. al., 2008; Du et. al., 2010). Both myocytes and adipocytes develop from the same pool of mesenchymal stem cells (Du et. al., 2010). Adipocytes form the site for intramuscular fat to accumulate and develop marbling in the offspring (Tong et. al., 2009). Adipogenesis begins around mid-gestation in ruminants and continues to birth (Feve, 2005; Gnanalingham et. al., 2005; Muhlhausler et. al., 2007). Adequate maternal nutrition during adipogenesis causes an increase in cells that differentiate into adipocytes creating offspring with more marbling potential (Du et. al., 2010). In sheep, overfeeding dams increases adipogenesis in fetal skeletal muscle (Tong et. al., 2008, 2009). This increase in intramuscular adipogenesis during fetal development causes the offspring to have a higher propensity to marble well, increasing their carcass value. Conversely, lambs from mid-gestation nutrient restricted dams matured to be fatter with a lower lean-to-fat ratio (Zhu et. al., 2006). Similar adaptations occur in the pig with small birth weight piglets being fatter and having decreased growth efficiency compared to normal birth weight

littermates (Wigmore and Stickland, 1983). Furthermore, DNA concentration per gram of peri-renal adipose tissue in steers born to dams nutrient restricted during early gestation was greater suggesting a greater number of adipocytes present or less lipid present in adipocytes (Long et. al., 2010). Programming effects on adipogenesis have the potential to decrease carcass quality. For example, steers exposed to maternal protein restriction during late gestation had lowered empty body fat percentage and 12^{th} rib fat thickness at slaughter (Summers et. al., 2015). Furthermore, tenderness measured by Warner-Bratzler shear force was decreased in steers from protein restricted dams (Summers et. al., 2015). In addition, small birth weight piglets, presumed to receive fewer nutrients than littermates, have an increase in collagen in skeletal muscle (Karunaratne et. al., 2005). Since collagen and connective tissue in meat are the major contributors to meat toughness, carcasses with higher amounts of collagen in the muscle are less valuable (Du et. al., 2010).

In beef cattle production systems, the incidence of nutrient restriction is significant due to poor forage availability and lack of supplementation, especially during early- to mid-gestation (Du et. al., 2010). Initial Average Daily Gain (ADG) tended to be less for steers from dams that underwent late gestational protein restriction, however, overall ADG in the feedlot phase was unaffected by protein content of maternal diets (Summers et. al., 2015). Conversely, early to mid-gestational protein supplementation increases lean muscle growth and lean-to-fat ratio in offspring (Du et. al., 2010). Furthermore, grazing cows, during mid-gestation, on improved pastures with higher crude protein content than native pastures, increases adipocyte numbers, weaning

weight, live weight at slaughter, carcass weights, meat tenderness, subcutaneous fat, and 12th rib back fat in steers (Underwood et. al., 2010). These data suggest that some negative effects of fetal programming can be alleviated by strategic nutrient supplementation of dams allow producers to optimize performance of offspring in their herd.

Future programming directions for the cattle industry

Although fetal programming has been investigated in many different aspects of fetal development and offspring performance, the causes of differences in results within each study are still unknown. Future studies need to focus on fetal programming impacts on carcass characteristics of feeder steers and heifers as well as impacts on heifer and cow reproductive performance and longevity. Furthermore, strategies to alleviate the negative impacts of nutrient restriction or other causes of programming need to be developed in order to increase production efficiency, animal health, and profitability of the different sectors of the cattle industry.

Structure and development of the ruminant placenta

The synepitheliochorial placenta of ruminants

The placenta is required for fetal survival and growth with the main function being to create a large surface area for exchange of nutrients and waste products across the epithelial barriers (Dunlap et al., 2015; Bazer et al., 2015). Ruminants have a synepitheliochorial placenta which is structurally and functionally different than for other livestock species (Green et al., 1998). Placenta cells fuse with uterine epithelial

cells to form a syncytium in addition to having both cross-current and counter-current blood vessels which contributes to efficient oxygen exchange from maternal to fetal circulations (Leiser et. al., 1997). In order to enhance substance exchange, hormonal control and anchor the maternal and fetal tissues, the ruminant placenta contains functional units called placentomes that include a maternal portion, the caruncle, and a fetal portion, the cotyledon (Leiser et. al., 1997). Placentomes are the main sites of nutrient and waste exchange across the placenta (Green et al., 1998).

Early development of the embryo and trophectoderm

The bovine placenta initially develops from cells in the outermost layer of the expanded blastocyst termed the trophectoderm. The outer trophoblast cells along with the somatic mesoderm become the chorion. The allantois is a fluid filled sac that develops from the hindgut of the fetus. The allantois will expand within and fuse with the trophectoderm/chorion to establish the highly vascularized, chorioallantoic placenta. In addition, the amnion is a sac that surrounds the fetus, fills with amniotic fluid and fuses with the chorion. The amnion and chorioallantois are the fetal membranes that enclose the fetus inside the uterus (Figure 1).

Figure 1. Bovine fetus within amnion and allantois with cotyledons present at approximately GD70. Reprinted with permission from Schlafer et al. (2000).

At GD33, the chorioallantois attaches to the endometrium (Schlafer et al., 2000) and initiates development of functional units, the placentomes. The chorioallantois develops cotyledons which attach to the caruncles on the endometrium. This attachment occurs by villous projections from the cotyledons occupying crypts in the caruncle, and

functions to greatly increase surface area for the exchange of nutrients, gases, and waste (Schlafer et al., 2000; Leiser et al., 1997).

Interdigitation in placentome

The placentome is formed by interdigitation of the caruncular crypts and the cotyledonary villi which create a juxtaposition of maternal and fetal blood vessels (Figure 2) (Leiser et. al., 1997).

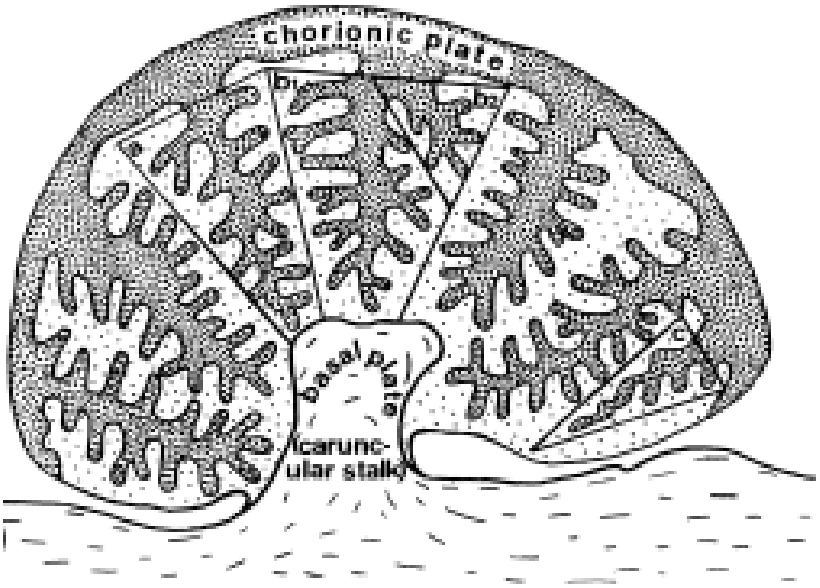

Figure 2. Drawing of the interdigitation of the caruncular crypts and cotyledonary villi of the bovine placentome. Tall conical villous trees are present during late gestation (a). Villous trees resembling Christmas trees are present in the full-grown stage (b_1) and the budding stage (b_2) of mid-gestation. Reprinted with permission from Leiser et al. (1997).

As pregnancy progresses, the microvasculature within the placentome increases to meet the growing demands of the fetus for the exchange of nutrients, waste, and gases (Lawn et al., 1969; Dunlap et. al., 2015).

Binucleate Cell Migration

Nutrient and waste transport between the dam and fetus is dependent on the cellular transport of products, as well as blood flow through the uterus and placenta. During early placentation, a continuous layer of trophectoderm cells forms across the entire surface of the chorioallantois including mononucleated and binucleated trophectoderm cells (Wallace et al., 2015). Within mononucleated trophoblast cells that constitute about 80% of the trophoblast cell numbers, two populations that are phagocytic in nature (Green et al., 1998; Schlafer et al., 2000). These cells function to phagocytize maternal erythrocytes and line the bases in between the cotyledonary villi on the fetal side of the placentome, as well as over the openings of the endometrial glands in the interplacentomal regions of the chorioallantois. Binucleated trophectoderm cells make up about 20% of the total trophectoderm cell population (Green et al., 1998; Schlafer et al., 2000). A fetal-maternal syncytium is formed by the binucleated trophoblast cells (BNCs) and uterine epithelial cells (Green et al., 1998). BNCs appear around GD21 by arising from mononucleated trophoblast cells through mitotic polyploidy (Wooding and Wathes, 1980; Wallace et al., 2015). BNCs contain two nuclei and large amounts of granules that include a myriad of products such as bovine placental lactogen (CSH1) and pregnancy-associated glycoproteins (PAGs) (Wooding, 1992). The binucleate cells also migrate into the maternal epithelium in the caruncular crypts and

the interplacentomal areas and fuse with uterine epithelial cells to release their cytoplasmic granules creating a trinucleated hybrid cell (Wooding and Wathes, 1980; Wooding, 1992). Binucleate cell degranulation occurs over the last 10 days of pregnancy corresponding to a systemic rise in PAGs during that period of pregnancy. However, the role of bovine PAGs and other products of trophectoderm cells is not fully elucidated (Schlafer et al., 2000). Binucleate trophectoderm cells produce and secrete hormones and growth factors such as progesterone, CSH1, and PAGs, in addition to other protein hormones vital for fetal survival and growth (Reimers et al., 1985; Duello et al., 1986; Roberts et al., 1995; Green et al., 2000; Schlafer et al., 2000; Pfarrer et al., 2006; Hashizume et al., 2007; Wallace et al., 2015). When *CSH1* is knocked down in sheep, IUGR results with total fetal, fetal liver, and placenta weights decreased by 52%, 41%, and 32% respectively (Baker et al., 2016). These data suggests that *CSH1* from binucleated cells is vital for placental and fetal development. Although binucleate trophoblast cells have the ability to produce progesterone, concentration of progesterone in fetal blood remain low. Therefore, binucleate cells must not produce progesterone until migration from the cotyledon into the caruncle has occurred (Reimers et al., 1985). The number of BNCs decreases rapidly one or two days before parturition (Wooding, 1992). The main developmental events of the bovine placenta are displayed in a timeline (Figure 3).

Figure 3. Timeline demonstration of the various key developmental events of the bovine placenta during gestation.

Size/growth of placentomes relative to location

The placentomes closest to the fetus develop earlier in gestation than those more peripheral to the chorioamnion (Leiser et. al., 1997). At GD48, all placentomes are located in the gravid uterine horn (Steyn et. al., 2001) however, placentomes develop in the contralateral uterine horn as gestation continues, ultimately having four rows of placentomes line each uterine horn (Leiser et. al., 1997). Placentome size and length are associated with gestational age of the fetus (Adeyinka et al., 2014). Placentome length

increases as gestation progresses independent of breed type, age of the cow, or herd being sampled (Rotta et. al., 2015). Placentomes in the gravid uterine horn ipsilateral to the ovary bearing the corpus luteum, develop to a larger size than those in the contralateral uterine horn or at the tip of the ipsilateral uterine horn (Schlafer et al., 2000; Leiser et al., 1997). Placentomes in the non-gravid uterine horn are smaller and fewer in number than those in the gravid uterine horn (Schlafer et. al., 2000). Placentomes in the contralateral uterine horn have greater variation in weight than those in the ipsilateral uterine horn. However, placentome size varies greatly due to among cow variation making the use of placentome measurements an inconsistent measure of gestational age (Adeyinka et al., 2014). Furthermore, there is no evidence that development of placentomes in the non-gravid uterine horn is affected by development of placentomes in the pregnant uterine horn (Laven and Peters, 2001). The number of placentomes and the mean weight or size (surface area) of the placentomes is unrelated. However, mean weight and size of placentomes does increase throughout gestation (Laven and Peters, 2001). Unlike the sheep, whose placentomes cease to grow at GD90 , bovine placentomes continue to grow throughout gestation with the weight increasing significantly from GD60 to 190 (Laven and Peters, 2001). The continued growth of placentomes throughout gestation could potentially result in the exchange of nutrients and waste between the dam and fetus to be more adaptable to environmental changes through compensatory growth (Laven and Peters, 2001). The proportion of caruncular tissue in placentomes increases as gestation advances regardless of location in the uterus (Laven and Peters, 2001). Similar to the ovine placenta, the number of placentomes in

cattle, does not significantly increase throughout gestation to account for the increased demands of the fetus. Instead of the weight, size, or number of placentomes increasing in late gestation, vascular development, particularly capillary bed volume, increases to meet increased fetal demands for oxygen and nutrients (Leiser et al., 1997).

Angiogenesis and blood flow

Angiogenesis in the placentome and factors affecting angiogenesis

The structure of the placental vasculature changes during gestation to meet the increasing demands of the fetus. Nutrient and waste exchange through the placenta is dependent on uterine and umbilical blood flows which are ultimately dependent on the presence of adequate vascularization and vasodilation (Vonnahme et al., 2007). Angiogenesis, the formation of new blood vessels, in the placenta and vasodilation of blood vessels are vital for increasing blood flow that is directly correlated with rate of exchange of nutrients and gases across the placenta in support of fetal development. Poor placental vascular development is a large contributor to low birth weight and in more severe cases, embryonic/fetal deaths in cattle and sheep (Reynolds et al., 1992). Villous trees develop and extend from the fetal chorionic plate to the maternal basal plate. In the budding stage, the villous trees grow on the periphery of the placentome. In mid-gestation, the villous trees resemble Christmas trees with the end closer to the chorionic plate being wider. In late gestation, tall conical shaped villous trees are present (Leiser et al., 1997). The base of these villous trees is connected by a main stem artery that develops from the chorioallantoic arterial system. Several stem veins form a tube-

like system around the stem artery running parallel to it. Unlike the stem artery, the stem veins are connected by inter-venous bridges, slightly convoluted, and variable in diameter. After GD170, the bovine placenta is fully formed, but villous trees continue to develop in the center of the placentome and growth of the placentome occured on its periphery to meet the growing needs of the fetus for oxygen and nutrients (Leiser et al., 1997). Nutrient and waste exchange across the placenta depends on the surface area of endothelial cells of capillaries in the cotyledons of the chorioallantois and inner vascular endothelial surface area of the caruncles. The increase in surface area of these areas enhances the rate of exchange of nutrients and gases. The vasculature of the placentomes includes supplying and working vessels. Supplying vessels include the villous trees that transports blood to the capillaries. Arteries and arterioles found in the villous trees run very straight from the chorionic plate to the terminal ends of the villi. Veins and venules are less straight in their position in the placentome, but still function in transport of nutrients and gases due to their thinner walls and more peripheral location (Leiser et al., 1997). Working vessels mainly consist of capillaries located in the peripheral or terminal villi that are very close to the maternal tissue or caruncles. The working vessels are the site of exchange of nutrients and gases and increase in surface area in the second half of gestation. During mid- to late- gestation in cattle, the extraction of oxygen by the uterus only increases 0.4 fold while uterine blood flow increases 4.5 fold (Reynolds et al., 2009). The design and function of the vasculature in the bovine placentome is intricate and vital for adequate transport of nutrients and gases from dam to fetus and, therefore, fetal growth and development. Poor vascular

development during early pregnancy may be associated with poor uterine and umbilical blood flow resulting in the fetus receiving an inadequate supply of nutrients. Inadequate nutrients can cause abortion and embryonic/fetal loss, poor placental formation, and altered fetal growth including IUGR (Grazul-Bilska et al., 2010).

Vascular Endothelial Growth Factor Alpha (VEGFA)

VEGFA functions in the placentome to regulate steroidogenesis, placental growth and function, proliferation and differentiation of cells, and angiogenesis. (Pfarrer et al., 2006; Campos et. al, 2010; Sousa et al., 2012). VEGFA is the most important factor in regulating angiogenesis and vascularization in the bovine placentome (Sousa et. al., 2012). Greater levels of VEGFA increase vascularization of placentomes and, therefore, increase blood flow and nutrient transfer from the dam to the fetus (Rotta et al., 2015). Pfarrer et al. (2006) suggests that VEGFA has an autocrine function on trophoblastic giant cell migration during placental establishment. Before implantation occurs, VEGFA is localized in the mononuclear trophoblast cells and the trophoblastic giant cells, as well as the uterine epithelium. As early placentation occurs, VEGFA is localized more in fetal and maternal vascular structures and less in the mononuclear trophoblast cells. During late gestation, VEGFA is located more extensively in the trophoblastic giant cells and uterine epithelium and is no longer present in the maternal connective tissue (Pfarrer et al., 2006). Expression of VEGFA mRNA has a positive relationship with capillary density and capillary size in the caruncle as well as cotyledonary branching, which has a positive relationship with blood flow (Borowicz et. al., 2007; Reynolds et al., 2009).

FMS-like Tyrosine Kinase(FLT1)

FLT1, the receptor for VEGFA, is abundantly expressed in trophoblast giant cells, as well as fetal and maternal endothelia in the developing bovine placentome (Pfarrer et al., 2006). At GD 20, *FLT1* expression is significantly greater than expression in non-pregnant ewes and continues to increase from GD 26 to GD 30 (Grazul-Bilska et al., 2010). The expression of *FLT1* is positively correlated with the growth of caruncular vasculature as well as degree of branching of cotyledonary vasculature (Borowicz et al., 2007).

FGF2 (Fibroblast Growth Factor 2)

FGF2 functions to regulate development and differentiation and is particularly involved in angiogenesis and stimulation of proliferation and differentiation of trophoblastic giant cells (Pfarrer et. al., 2006). FGF2 expression is highly correlated with the degree of cotyledonary vasculature branching (Borowicz et al., 2007). FGF2 is expressed throughout pregnancy in the vascular endothelial cells, endometrial cells, and trophoblast cells (Campos et al., 2010). The level of expression of FGF2 mRNA has a strong negative relationship with capillary density in the caruncle (Reynolds et al.,2009). In addition, FGF2 induces vasodilation which increases the volume of blood flow to allow for increased feto-maternal exchange (Pfarrer et al., 2006). Vasodilation reduces peripheral resistance to blood flow and is always associated with an increase in rate of blood flow through a tissue or organ.

ODC1 (Ornithine Decarboxylase)

Ornithine decarboxylase (ODC1) plays an important role in the conversion of amino acids to polyamines. Arginine is first converted into ornithine by the enzyme, arginase. Then, *ODC1* is responsible for converting ornithine into the polyamine, putrescine (Bazer et al., 2015). Putrescine can then be converted into spermidine by spermidine synthase or spermine by spermine synthase (Kwon et al., 2003). Putrescine, spermidine, and spermine are necessary for implantation, placentation, angiogenesis, and embryonic and fetal growth (Bazer et al.,2015; Hussain et al., 2017). When ODC1 is inhibited by the administration of DL-a-difluoromethyl ornithine (DFMO) during early pregnancy in the rat, they abort and pups suffer from IUGR. Furthermore, DFMO administration and therefore, the reduction of polyamine synthesis through the ODC1 pathway caused reduced total fetal, placental, fetal brain, and fetal liver weights (Ishida et al., 2002). This suggests that OCD1 is vital for the synthesis of polyamines for placental and fetal growth.

Nutrient Transport in the Bovine Placenta

One of the rate limiting factors for delivery of nutrients to the developing fetus is the expression and activity of specific glucose and amino acid transporters (Fowden et al., 2006). The expression and activity of these transporters can be affected by environmental factors such as nutrition, hypoxia, heat stress, and exposure to hormones (Dunlap et al., 2015). These factors can alter glucose or amino acid levels and rate of transfer to the fetus which in turn, will lead to adaptions by the fetus in order to survive and continue to develop. Amino acids are essential for growth of the fetus, serving as

building blocks of protein synthesis, antioxidants, hormone regulators, cell signaling molecules and acting as precursors for many non-protein substances. For example, nitric oxide is a product of arginine catabolism. Nitric oxide functions to regulate placental angiogenesis as well as feto-placental blood flow during pregnancy (Reynolds and Redmer, 2001). Furthermore, polyamines, which are molecules synthesized from ornithine, have various physiological roles including regulation of gene expression, DNA and protein synthesis, cell proliferation, cell differentiation, and cell function. Additionally, downregulation of amino acid transporters in the placenta occurs before the incidence of IUGR offspring in pregnant rats fed a low proteindiet. Furthermore, placental weights were unaltered by dietary protein at GD15-GD19, but were reduced at GD21 in rats fed low protein diets that decreased the activity of placental system A transporters. Those data suggest that down-regulation of these transporters is a contributor to the incidence of IUGR (Jansson et al., 2006).

SLC2A1 (Solute Carrier Family 2 Member 1)

SLC2A1 is the major glucose transporter in the ruminant placenta (Wooding et al., 2005). *SLC2A1* is highly expressed between the maternal and fetal endothelia on the innermost and outermost membranes of the placenta (Dunlap et al., 2015). SLC2A1 transports glucose from maternal to fetal circulation through facilitated diffusion (Lucy et al., 2012). In sheep, *SLC2A1* expression increases throughout gestation and peaks at GD120-140 (Dandrea et al., 2001). This glucose transporter is vital for survival of the fetus, as the placenta and fetus have very limited capacity gluconeogenesis (Battaglia and Meschia, 1978; Lucy et al., 2012).

High Affinity Cationic Amino Acid Transporter 1(SLC7A1)

SLC7A1 is a cationic amino acid transporter located on the basal membrane in the placentome (Zhang et al., 2015). The expression of *SLC7A1* is consistent throughout pregnancy in the sheep (Dunlap et al., 2015). SLC7A1 is vital for fetal development as it transports arginine, histidine, lysine, and ornithine into the fetal circulation (Wu, 2013).

Hormonal Action in the Bovine Placenta

Pregnancy Associated Glycoprotein 1(PAG1)

Pregnancy associated glycoproteins (PAGs) make up a large family with 21 bovine specific glycoproteins identified and abundantly expressed by the placenta (Green et al., 2000; Wallace et al., 2015). These PAGs can be divided into two groups based upon tissue expression. The first group is expressed in the outer epithelial layer of the placenta (trophectoderm) while the second group is expressed largely in the binucleate cells. *PAG1*, if present at all, is weakly expressed by binucleated cells at GD25, however, this glycoprotein is present in mid- and late-gestation (Hashizume et al., 2007). As the pregnancy nears terms, *PAG1* expression again declines to negligible levels (Green et al., 2000). PAGs are thought to have arisen from a group of aspartic proteinases however, bovine PAG1 lacks one of the conserved residues making PAG1 unable to act as a proteolytic enzyme (Xie et al., 1991; Green et al., 1998; Wallace et al., 2015). Instead, there are two theories of the function of PAG1 on placental function and fetal development. First, it is suggested that PAGs have an immune function (Wallace et al., 2015). Endometrial cells treated with PAG1, had a decrease in proliferation of hematopoetic cells (Hoeben et al., 1999). Furthermore, the activity of immune cells in

the cow is lowest at the end of pregnancy and duringparturition (Kehril et al., 1989; Saad et al., 1989; Hansen et al., 2013; Wallace et al., 2015). If PAG1 has immunosuppressive or immunoregulatory functions, they could be contributing to the suppression of the maternal immune system near parturition (Wallace et al., 2015). The second potential function of PAG1 is to exhibit a luteotrophic role. PAG1 treatment of luteal cells increases secretion of prostaglandin E_2 (PGE_2) which has luteotrophic and antiluteolytic functions to prevent spontaneous luteolysis in bovine pregnancy (Del Vecchio et al., 1996; Weems et al., 1998a; Weems et al., 1998b; Wallace et al., 2015). If PAG1 did possess a luteotrophic function, an increase in progesterone would be logical and there is evidence of increased concentrations of progesterone in culture media when PAG1 was administered to luteal cells (Del Vecchio et al., 1996; Weems et al., 1998b).

Placental Lactogen(CSH1)

Placental lactogen is synthesized solely by binucleated cells (Green et al., 2000; Ravelich et al., 2004). Placental lactogen is not expressed during implantation, but is lowly expressed in the placentome during early placentation and peaks in mid-gestation before plateauing to term (Ravelich et al., 2004). The expression of mRNA for placental lactogen is localized to trophoblast cells in the placentome from GD50-150 (Ravelich et al., 2004). Placental lactogen decreases in the inter-placentomal region of the placenta as gestation progresses (Hashizume et al., 2007). Placental lactogen functions to form the fetomaternal syncytium, and CSH1 protein retention is greater in pregnancies with poor attachment such as nuclear transfer derived pregnancies and is positively correlated with placental and fetal weights (Ravelich et al., 2004). More specifically, this molecule has

functional roles associated with luteotropic activity, fetal growth, lactogenesis, nutrient partitioning, and angiogenesis of the placenta (Ravelich et al., 2004; Hashizume et al., 2007).

Estrogen Receptor(ESR1)

Estrogen is the primary regulator of uteroplacental blood flow as it upregulates angiogenic and vasoactive factors (Reynolds et al., 2009). Cotyledons are the main source of placental estrogens (Evans and Wagner, 1981; Larsson et al., 1981; Gross and Williams, 1988; Hoedemaker et al., 1990; Hoffman and Schuler, 2002). Estrogen receptors are located in caruncular stromal cells, caruncular epithelial cells, and caruncular capillary pericytes (Hoffman and Schuler, 2002). As concentrations of estrogen in the caruncle decrease from GD150-270, proliferation of caruncular stromal cells also decreases (Hoffman and Schuler, 2002). Estrone-3-sulfate is the major placental estrogen and it is detected in maternal plasma as early as GD33 but does not increase until GD70-100 and then plateaus between GD265 and parturition (Hoffman and Schuler, 2002). Concentrations of placental estrogen in plasma greatly exceeds those of ovarian origin and these higher concentrations are maintained over several months (Hoffman and Schuler, 2002). As the animal nears parturition, placental estrogen is markedly increased to stimulate myometrial contractions (Hoffman and Schuler, 2002).

Insulin-like Growth Factor 1(IGF1)

The glucose-IGF1 axis has a major role in stimulating cell division in the fetus (Gluckman, 1995). The primary endocrine regulator of fetal growth in late gestation is IGF1 (Wali et al., 2012). IGF1 stimulates uptake of substrates, promotes cellular mitosis

differentiation, and migration, as well as inhibits protein degradation (Harding et al., 1994; Han and Carter, 2000; Ravelich et al., 2004). Furthermore, the bovine placenta is an active regulator of circulating concentrations of IGF1 in the fetus (Bauer et al., 1998; Ravelich et al., 2004). Intra-amniotic supplementation of IGF1 in IUGR sheep fetuses caused an increase in their growth, as well as increases in expression of SLC2A1 and amino acid transporters in the placenta. While IGF1 is absorbed intact by intestinal epithelia of the fetal gut when administered in the amnion (Bloomfield et al., 2002), concentrations of IGF1 in plasma of the fetus do not increase, suggesting that IGF1 supplementation indirectly increases fetal growth. Alternatively, IGF1 may be bound to its receptors on cells of the fetus immediately or bound to an IGFBP (Wali et al., 2012).

Insulin-like Growth Factor-binding Protein-3(IGFBP3)

Insulin-like growth factor-binding protein-3 (IGFBP3) regulates the bioavailability of IGF1, and also mediates inhibition and enhancement of growth, and initiation of apoptosis (Ravelich et al. 2004). IGFBP3 is the most abundant IGF1 carrier protein, binding 95% of IGF1 in bovine circulation (Wettaerau et al., 1999; Ravelich et al., 2004). *IGFBP3* is expressed by caruncular stroma, uterine luminal epithelium, myometrium, and endothelial cells found in the maternal blood vessel walls where it plays a role in angiogenesis (Renfree, 1982; Peterson et al., 1998).

Impacts of nutrition on placental development

Effects of nutrient restriction on placental size

Although an indirect measure of function, the size of the placenta is strongly associated with fetal size at birth (Godfrey and Barker, 2000). Early gestational nutrient

restriction combined with adequate nutrition during mid- and late-gestation results in an increase in size of the placenta. Conversely, females fed an adequate diet during early gestation and then restricted in later gestation had a reduced placental size (Robinson et al., 1994; Godfrey et al., 1996; Lumey, 1998; Perry et. al., 1999). In addition, nutrient restricted cows that produced IUGR calves had reduced cotyledonary weights and a tendency for a reduction in placentomal surface area compared to nutrient restricted cows that produced non-IUGR calves (Long et al., 2009). Nutrient restriction during both early- and mid-gestation reduces caruncular, cotyledonary, and fetal weights (Vonnahme et. al., 2007). Placental growth and size depends on severity of maternal nutrient restriction and the period of nutrient restriction during pregnancy.

Effects of nutrient restriction on placental angiogenesis

Maternal nutrient restriction affects various parameters of placental development, which impacts blood flow in the placentome and therefore, the transfer of gases and nutrients. The transfer of highly permeable substances such as oxygen and carbon dioxide from maternal to fetal circulations depends on blood flow, as these gases are transferred through simple diffusion (Zhang et. al., 2015). Vascularity of placentomes is impacted by gestational nutrient restriction which may result from altered expression of angiogenic factors. For example, when early- to mid-gestational nutrient restricted cows are fed a diet above maintenance to reach similar body condition score (BCS) as control cows by GD250, capillary number density, capillary cross-sectional area density, and capillary surface density in cotyledons are decreased suggesting decreasedblood flow in

the placenta (Vonnahme et. al., 2007). Similarly, in sheep, a 14% decrease in capillary area density in caruncles occurred in nutrient restricted pregnancies (Alexander, 1964). Cows that were nutrient restricted from GD30 to GD125, had greater expression of FLT1 that could be compensating for maternal diet as capillary density, capillary cross-sectional area, capillary surface, and average cross-sectional area per capillary at GD125 were unaffected (Vonnahme et. al., 2007). Furthermore, VEGFA and FGF2 expression was greater in Gyr x Holstein cows fed 100% of energy requirements compared to cows fed 190% of energy requirements at GD268 (Rotta et. al., 2015). These results suggest that expression of angiogenic factors has an inverse relationship with nutrient availability during gestation and is a compensatory mechanism to promote fetal survival and growth.

Effects of nutrient restriction on placental nutrient transporters

Nutrient transfer of glucose and amino acids from maternal to fetal circulation depends on abundance, localization, and level of activity of nutrient transporters (Barry and Anthony, 2008; Zhang et al., 2015). Increased expression of nutrient transporters in the placenta can be a compensatory mechanism for increasing efficiency of nutrient delivery to under-developed or small placentas (Zhang et al., 2015). Glucose is transported across the placenta through facilitated diffusion (Zhang et al., 2015). In sheep, maternal nutrient restriction does not affect the expression of SLC2A1 in the placenta (McMullen et al., 2005). However, glucose moves down its concentration gradient from maternal to fetal circulation and low maternal glucose levels due to maternal nutrient restriction do not provide adequate glucose for transfer to the fetal

circulation (Lucy et al., 2012). Nonetheless, the IUGR fetus can compensate for the decrease in concentrations of glucose in the maternal circulation by increasing the concentration gradient which accelerates the rate of glucose transfer per gram of placental tissue (Owens et al., 1987a,1987b; Marconi and Paolini, 2008). Additionally, amino acids are actively transported using the charge from sodium ions to exchange for the charge of amino acids (Zhang et al., 2015). Results to evaluate the effects of nutrient restriction on amino acid transport through the placenta have been inconclusive with some studies reporting reduced umbilical amino acid uptake in severe IUGR (Regnault et al., 2007,2013) while others report no reductions (Anderson et al., 1997; Paolini et al., 2001). Arginine is essential for fetal growth as it is a substrate for synthesis of nitric oxide (NO) and polyamines (Lassala et al., 2011). Maternal protein restriction in pigs causes a 21-25% decrease in concentrations of arginine in fetal plasma, allantoic fluid, and placenta (Wu et al., 1998). While *SLC7A1* expression is unaffected by maternal nutrient restriction in sheep (Dunlap et al., 2015), decreased availability of amino acids ultimately leads to a reduction in concentrations of amino acids and polyamines in amniotic fluid, allantoic fluid, and fetal plasma. Arginine supplementation in ewes gestating multiple fetuses, increased birth weights of quadruplets, but not twins or triplets, in addition to increasing survival rates of lambs (Lassala et al., 2011). Arginine supplementation during pregnancy is a potential treatment to diminish the effects of IUGR due to uterine crowding in multi-fetus pregnancies. Another potential therapy to alleviate the impacts of maternal nutrient restriction is the administration of sildenafil citrate (Viagra), as it increases fetal weight, as well as concentrations of amino acids and

polyamines in amniotic, allantoic, and fetal umbilical vein serum (Satterfield et al., 2010).

Effects of nutrient restriction on placental hormonal action

The glucose-IGF1 axis is a major contributor to matching growth of the fetus to maternal nutrient supply (Gluckman, 1995). Reduced expression of *IGF1* contributes to reduced cell proliferation and fetal growth. Lowered expression of *IGF1* in pregnancies with a deficit in maternal nutrient availability may be a mechanism to prevent fetal loss which causes long-term fetal programming (Godfrey, 2002). IGF1 supplementation can alleviate some of the negative impacts of IUGR by increasing placental uptake of glucose and expression of *SLC2A1* and *SLC7A1* mRNAs (Gluckman and Harding, 1997; Harding et. al., 1997; Wali et. al., 2012). Expression of *SLC7A1* mRNA increased by 57% in placentas of IGF1 treated IUGR pregnancies making its expression similar to control pregnancies (Wali et al., 2012). Continued research is needed to elucidate the effects of nutrient restriction on PAGs, placental lactogen, and estrogen functions in the placenta.

CHAPTER III

RELATIONSHIP BETWEEN PLACENTOME LOCATION AND GENE EXPRESSION IN PREGNANT HEIFERS

Introduction

The traditional cow calf production system is susceptible to periods of gestational nutrient restriction and excess. Climatic conditions such as drought and seasonal growing patterns contribute to varying availability and quality of forages (Reynolds et. al., 2010). Furthermore, increased nutritional demands for growth are necessary for pregnant heifers and young cows compared to mature cows (Reynolds et. al., 2010). In turn, nutrient restriction increases calf morbidity and mortality, while decreasing ovarian antral follicle counts, initial ADG, tenderness, and birth weights of offspring, depending on the duration and severity of maternal nutrient restriction (Corah et al., 1975; Rae et. al., 2001; Micke et al., 2010; Summers et al., 2015). These negative impacts can decrease efficiency, increase cost of production, and decrease profitability throughout sectors of the beef industry. Proper placental development is vital for adequate blood flow, nutrient and waste exchange, and hormonal action (Bazer et al., 2015; Dunlap et al., 2015). Unlike species with a centralized discoid placenta, species with a cotyledonary placental type exhibit wide variations in both size and location in relationship to the developing fetus. Despite this variation, little is known regarding expression patterns of genes in placentomes at different locations within the gravid uterus of cattle. The objective of the present study was to determine variations in

expression of genes for angiogenic factors (*FGF2, ODC1, VEGFA,* and *FLT1),* nutrient transporters *(SLC7A1,* and *SLC2A1*), hormone action *(ESR1, IGF1,* and *IGFBP3),* and cotyledonary specific hormones *(CSH1* and *PAG1)* among placentomes relative to their location relative tothe fetus and corpus luteum. A secondary objective was to evaluate the impact of nutrient restriction on gene expression in placentomes.

Materials and Methods

Heifers

This study was conducted at Texas A&M University with all procedures and handling approved by the Institutional Animal Care and Use Committee. Ten crossbred heifers of predominantly Angus genetics with similar body condition, age, and frame size were utilized for this study.

Experimental Design

Pregnant heifers were maintained on adequate forage meeting their NRC requirements during early gestation then moved into pens with a Calan gate system (American Calan, Northwood, NH). From GD146 to GD244, five heifers received a hay diet to meet 100% of maintenance requirements, and five heifers were fed to meet only 70% of maintenance requirements. Total requirements for maintenance and pregnancy were estimated for each heifer and multiplied by 0.7 for those receiving the 70% diet. Mean body weight was calculated using individual weights from three consecutive days before the initiation of the experiment. The forage diet consisted of alfalfa hay (78.13% of DM), and wheat straw (21.87% of DM) with 16.49% CP, 2.07 Mcal/kg ME, and 1.22

Mcal/kg NE_m. Heifers were humanely euthanized at GD 270 using an overdose of phenytoin/pentobarbital (Beuthanasia-D, Merck Animal Health, Madison, NJ). The gravid uterus was removed and weighed. Following this, the fetus was removed from the uterus, weighed, and allantoic and amniotic fluids were drained. The empty uterus with the placenta intact was laid out with incisions made in four locations. Then, a single representative placentome was selected from a location on the antimesometrial side of the gravid horn; 1) central to the amnion, 2) over the chorioallantois immediately adjacent to the amnion, 3) the tip of the gravid horn, and 4) in the tip of the contralateral horn (Figure 4).

a

b

Figure 4. Location of incision and placentome removal from the gravid uterus and placentome appearance based on location. Figure 4a indicates the location of placentome removal from the ipsilateral and contralateral uterine horns (A- TIP, B- ALL, C- AMN, and D- CONTRA). Figure 4b shows the morphology of representative placentomes removed from the corresponding locations.

Placentomes were weighed, finely minced, snap frozen in liquid nitrogen, and stored at –80^{o}C until analyzed for gene expression. Genes encoding the expression of factors associated with angiogenesis, nutrient transport, and hormone action, as well as cotyledonary specific genes were evaluated using real time quantitative polymerase chain reaction (QPCR), as previously described (Wang et al., 2014). The following genes were analyzed for their importance in placental growth and function and fetal growth: *Basic Fibroblast Growth Factor (FGF2), Estrogen receptor alpha (ESR1), High Affinity Cationic Amino Acid Transporter 1 (SLC7A1), Insulin-like Growth Factor 1 (IGF1), Insulin-like growth factor binding protein 3 (IGFBP3), Ornithine Decarboxylase (ODC1), Placental Lactogen (CSH1), Pregnancy Associated Glycoprotein 1 (PAG1), Solute Carrier Family 2 Member 1(SLC2A1), Vascular Endothelial Growth Factor A (VEGFA),* and *Vascular Endothelial Growth Factor Receptor (FLT1). Beta Actin (B actin)* was used as the loading control.

RNA Extraction, cDNA Synthesis, and Real-Time Quantitative PCR Analyses

As previously described by Wang et. al. (2014), total RNA was isolated from bovine placentomes using Trizol (Invitrogen) according to the manufacturer's specifications. Concentrations of RNA were determined using Nanodrop, and quality of total RNA determined using a Bioanalyzer. Samples of total RNA were cleaned using an RNeasy Kit (Qiagen). The expression of mRNAs encoding for *CSH1, ESR1, FGF2, FLT1, IGF1, IGFBP3, ODC1, PAG1, SLC2A1, SLC7A1,* and *VEGFA* in bovine placentomes from differing regions within the uterus was determined using QPCR. Forward and reverse primer sequences as well as accession numbers are provided in

Table 2. By adding a dissociation curve step to the QPCR reaction and generating a standard curve from known quantities of bovine placentome cDNA, the specificity and efficiency of the primers were determined. Primers only amplifying a single product with an efficiency between 97.5% and 102.5% were used for analyses. First strand cDNAs were synthesized from 1µg of total RNA using Oligo (deoxythymidine) primers, and SuperScript II Reverse Transcriptase, according to manufacturer's specifications. Quantitative PCR was performed using the ABI Prism 7900HT system (Applied Biosystems) with Power SYBR Green PCR Master Mix (Applied Biosystems) as specified by the manufacturer. Each individual sample was run in triplicate using the following conditions: 50° C for 2 min, 95° C for 10 min, followed by 40 cycles of 95° C for 15 s and 60° C for 30 s. The threshold line was set in a linear region of the plots above the baseline noise, and threshold cycle (C_T) values determined at the cycle number at which the threshold line crossed the amplification curve. Bovine Beta Actin was used as the reference gene. The expression of *CSH1, ESR1, FGF2, FLT1, IGF1, IGFBP3, ODC1, PAG1, SLC2A1, SLC7A1,* and *VEGFA* mRNAs was calculated using the comparative C_T method.

Statistical Analysis

Gene expression data were analyzed using the PROC MIXED in SAS 9.4 (SAS Inst. Inc., Cary, NC). The model included effects of placentome location, diet, and location x diet interactions. Significance was established for all variables at $P \leq 0.05$, with $P \leq 0.10$ defined as a trend toward significance.

To evaluate variance due to heifer, diet, and placentome location, data were analyzed as a split plot design, with dietary treatments serving as the main plot effect with animal as the experimental unit, and location/sampling site serving as the subplot effect.

Results

Placentome weights were analyzed for differences among locations in reference to the fetus and results are summarized in Table 3. Placentomes located on the antimesometrial side of the gravid horn central to the amnion (AMN) (74.3 ± 7.6 g) and over the allantois immediately adjacent to the amnion (ALL) (75.7 ± 7.6g) were heavier ($P<0.05$) than placentomes located in the tip of the ipsilateral (TIP) (25.9 ± 7.6g) and contralateral uterine horns (CONTRA) (19.6 ± 7.6g).

Table 2 Primers utilized for quantitative real-time PCR analysis

Amplification Target	Forward/Reverse Primers (5'-3')[2]	Length of amplicon, bp	GenBank accession No[3]
VEGFA	GGGGCTGCTGTAATGACGAA	96	NM_001316955.1
	GCTGGCTTTGGTGAGGTTTG		
SLC7A1	CCTTCCAAATTCTCCGGGCT	148	NM_001135792.1
	AGGCAGAGCCCATGAGTAGA		
SLC2A1	CATTGTGGGCATGTGCTTCC	142	NM_174602.2
	AATCTCATCGAAGGTCCGGC		
PAG1	GCTTGTTCTGATGGCTGCAA	101	NM_174411.2
	ATGGCACCGATGAGCCTATG		
ODC1	TGCCTTCTATGTTGCGGACC	92	NM_174130.2
	TGACGGCATAAAAGGGGGTG		
ESR1	GCGGAATACGGAAAGACCGA	112	NM_001001443.1
	TTGGCAGCTCTCATGTCTCC		
CSH1	CTGCCGGATTCCCCTTCAAA	105	NM_001164321.1
	AACAGGGCTTCGTCAAATTCA		
FGF2	CCTACTCCTAGGCAATATGGTAAAT	96	NM-174056.3
	CAACCCACCTAGTCAGAGATTG		
Beta Actin	GCCCTGAGGCTCTCTTCCA	89	AF176419
	CGTCACACTTCATGATGAAATTGA		
FLT1	TGCGAAACCTCAGTGACCTC	152	NM_001191132.3
	GCTGCTTCCCGGTCCTAAAA		
IGF1	CAGCAGTCTTCCAACCCAAT	88	NM_001077828.1
	GAGATGCGAGGAGGATGTGA		
IGFBP3	ACAAAGGCGGGGATTTCTGA	113	NM_174556.1
	ACTTGTGATGCCTCTGGCAA		

Table 3. Average weights of placentomes from the gravid horn central to the amnion (AMN), and over the allantois immediately adjacent to the amnion (ALL), as well as placentomes located in the tip of the ipsilateral (TIP) and contralateral (CONTRA) uterine horns.

Placentome Location	**Mean Placentome Weight, g**
AMN	74.3 ± 7.6^{a}
ALL	75.7 ± 7.6^{a}
TIP	25.9 ± 7.6^{b}
CONTRA	19.6 ± 7.6^{b}

^{a,b} Means ± SEM within a measurement with different superscripts differ $P<0.05$

Expression of mRNAs for genes associated with angiogenesis were analyzed to identify differences in gene expressiondue to placentome location and maternal diet. There were no differences ($P > 0.10$) in expression of *VEGFA*, *FLT1*, *FGF2*, and *ODC1* mRNAs due to maternal diet or location of placentomes within the uterus (Figure 5).

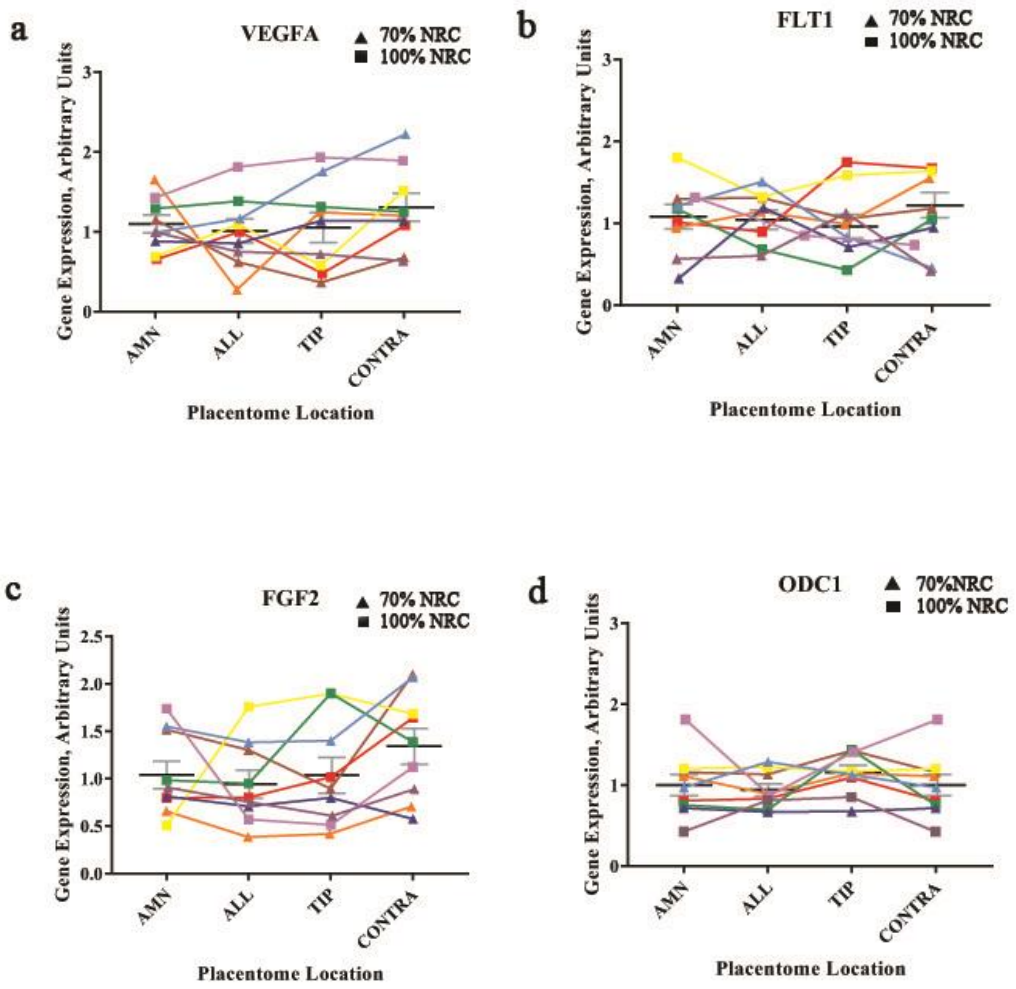

Figure 5. Expression of mRNAs for angiogenic factors in placentomes from heifers during late gestation. Data for each individual heifer is denoted by a unique color that remains consistent across placentome locations. There were no differences ($P > 0.10$) in expression of mRNAs for *VEGFA*, *FLT1*, *FGF2*, and *ODC1* due to placentome location or maternal diet. Legend: Placentomes were from the gravid horn central to the amnion (AMN), over the allantois immediately adjacent to the amnion (ALL), and the tips of the ipsilateral (TIP) and contralateral (CONTRA) uterine horns.

The expression of mRNAs for nutrient transporters were evaluated to identify effects of placentome location, diet, and location x diet interactions. Expression of mRNAs for *SLC2A1* and *SLC7A1* did not differ ($P > 0.10$) due to location of placentomes within the uterus or maternal diet (Figure 6).

Expression of mRNAs genes associated with hormonal action were quantified to evaluate differences among location of placentomes, maternal diet, and location x diet interaction. Expression of mRNAs for *IGF1, IGFBP3,* and *ESR1* was not different ($P > 0.10$) due to placentome location, maternal diet, or the interaction of placentome location and diet ($P > 0.10$) (Figure 7).

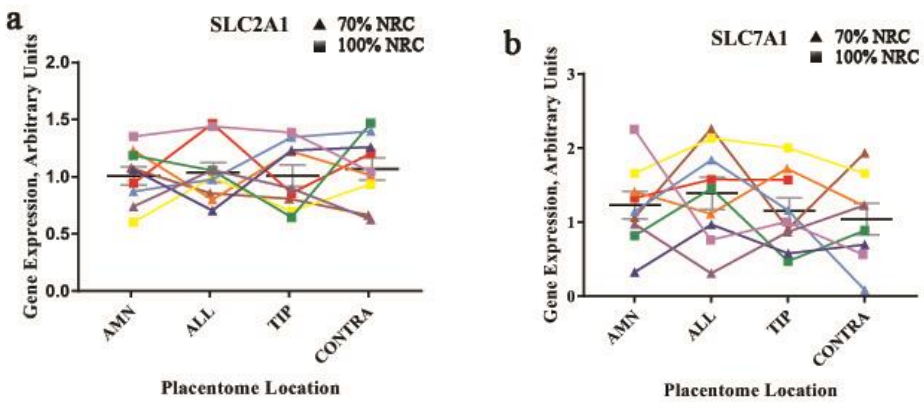

Figure 6. Expression of nutrient transporters by placentome location and diet for individual heifers is denoted by a unique color. Expression of mRNAs for angiogenic factors, SLC2A1 and SLC7A1, within placentomes from heifers in late gestation were unaffected ($P > 0.10$) by placentome location or maternal diet. Legend: Placentomes were from the gravid horn central to the amnion (AMN), over the allantois immediately adjacent to the amnion (ALL), and the tips of the ipsilateral (TIP) and contralateral (CONTRA) uterine horns.

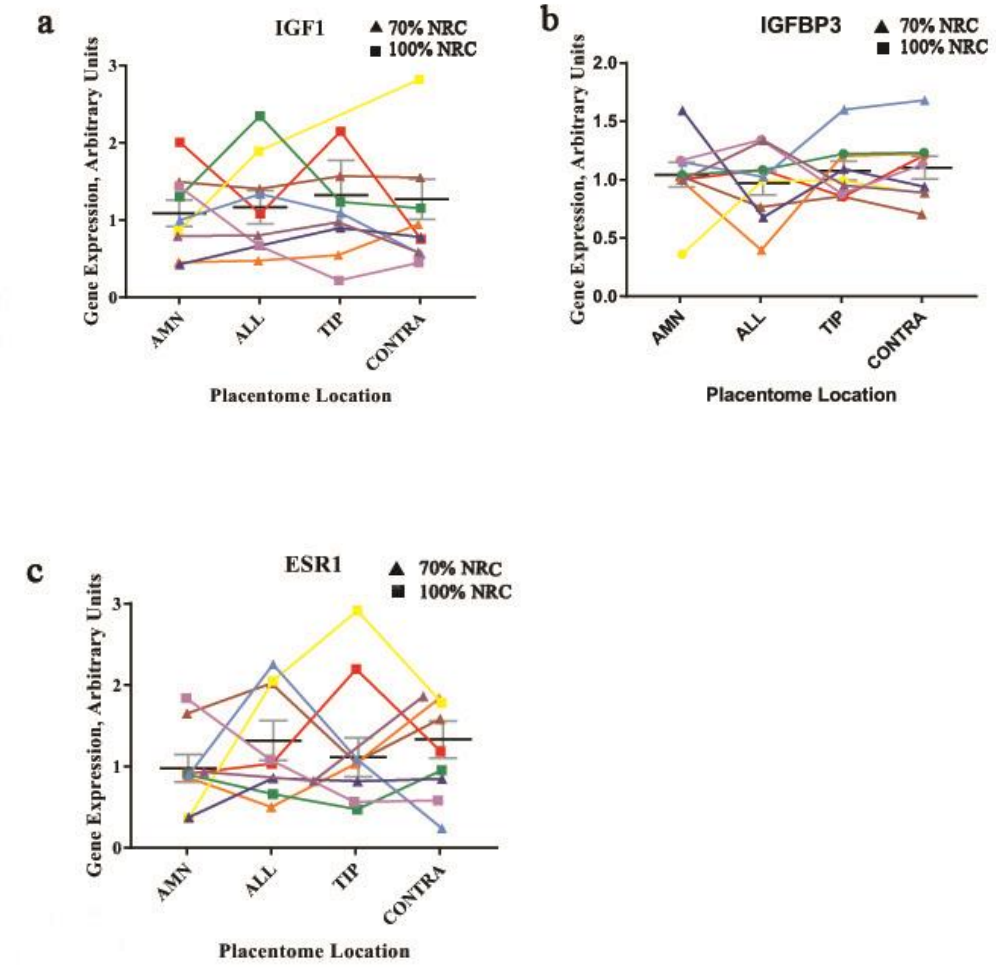

Figure 7. Individual heifer expression of mRNAs for hormone action due to placentome location and diet are denoted for each heifer by a unique color. There were no differences (P > 0.10) in expression of mRNAs for *IGF1*, *IGFBP3*, or *ESR1* due to placentome location or maternal diet. Legend: Placentomes were from the gravid horn central to the amnion (AMN), over the allantois immediately adjacent to the amnion (ALL), and the tips of the ipsilateral (TIP) and contralateral (CONTRA) uterine horns.

Expression of mRNAs for cotyledonary specific genes were analyzed to identify differences due to location of placentome within the uterus, maternal diet, or the interaction of location and diet. There were no differences (P > 0.10) in expression of mRNAs for *CSH1* and *PAG1* due to placentome location or maternal diet (Figure 8).

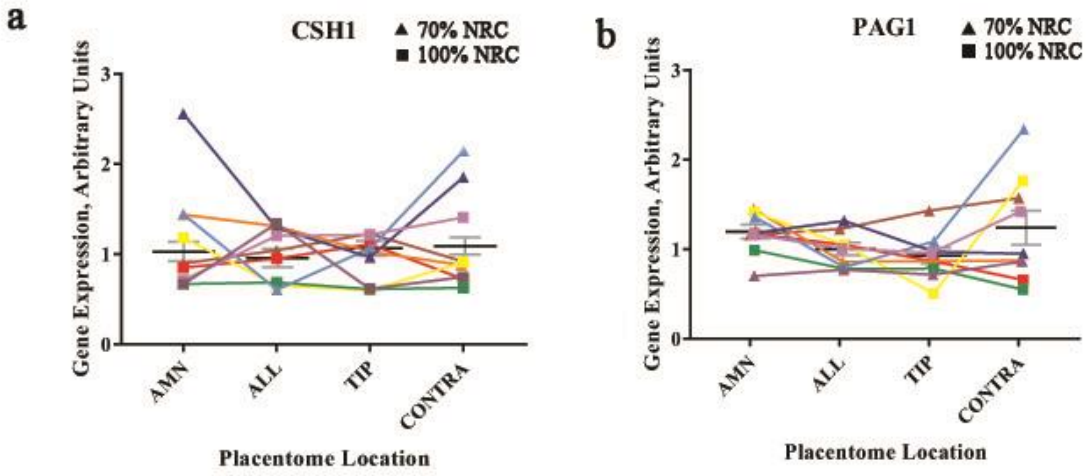

Figure 8. Expression of mRNAs for cotyledonary specific factors, *CSH1* and *PAG1*, across placentome location and diet is denoted by a unique color. There were no differences (P > 0.10) in expression of mRNAs for *CSH1* and *PAG1* due to placentome location or maternal diet. Legend: Placentomes were from the gravid horn central to the amnion (AMN), over the allantois immediately adjacent to the amnion (ALL), and the tips of the ipsilateral (TIP) and contralateral (CONTRA) uterine horns.

Discussion

Appropriate fetal growth is dependent upon development of a fully functional placenta. The ruminant placenta is characterized by 75-125 discrete units of high throughput nutrient and gas exchange, called placentomes. Sampling of these placentomes for subsequent molecular biology analyses has long been a tool of reproductive biologists to better understand placental development and function in relation to fetal growth. The emerging field of fetal programming has driven a need for sampling of placental tissues without compromising the pregnancy to allow for correlation between the uterine environment and the subsequent postnatal phenotype of the offspring. The present study is a first step in developing non-terminal methodologies for these retrospective analyses. Results of the present study indicate that expression of selected genes among placentomes does not differ due to the location of the placentomes within the gravid uterus.

To meet our objective of determining variation of gene expression within placentomes from different regions within the uterus we utilized a candidate gene approach. The chosen genes represent a variety of critical placental functions including, angiogenesis, nutrient transport, and hormonal signaling. As expected, the size of the placentomes varied greatly within the uterus and weights of the placentomes were greater when collected from regions over the amnion and over the allantois near the amniotic membrane compared to locations at the tips of either the placenta in either the ipsilateral or contralateral uterine horn. Despite these differences in weights, mRNA levels for the candidate genes did not differ due to location of the placentome.

Expression of *VEGFA* and its receptor *FLT1* functions to regulate placental angiogenesis and cell proliferation (Pfarrer et al., 2006; Campos et. al, 2010; Sousa et al., 2012) and is positively correlated with vascularization and increased capillary bed density in the placentome (Reynolds et. al., 2009; Rotta et al., 2015). Differences in *VEGFA* and *FLT1* expression were not present at GD270 suggesting that angiogenesis and cell proliferation is not impacted by location of the placentome or maternal diet. Furthermore, expression of *FGF2* mRNA was not different due to diet of the dam or location of the placentome in relation to the fetus. *FGF2* is associated with angiogenesis however, it has a strong negative correlation with capillary density in the placentome (Reynolds et al.,2009). While our analyses indicated that expression of genes for angiogenic factors did not differ among placentomes from differing locations, we cannot eliminate the possibility that there are histological differences between placentomes from differing locations. Expression of *ODC1* mRNA did not differ among placentomes or maternal diet suggesting that polyamine synthesis is unaffected in late gestation by placentome location or maternal diet. These results suggest that expression of angiogenic factors from smaller, non-centralized placentomes accurately represents expression of these angiogenic factors within the larger placentomes more central to the fetus.

Nutrient transporters are critical in the exchange of amino acids for protein synthesis and glucose for various metabolic pathways critical to the fetus, as well as biochemical processes for generation of urea (arginine-dependent urea cycle) for transfer to the dam for excretion. Maternal protein restriction results in alterations in expression of placental nutrient transporters and these alterations precede the development of IUGR

(Jansson et al., 2006). In the present study, expression of the glucose transporter *SLC2A1* and the cationic amino acid transporter *SLC7A1* was unaffected by location of placentomes within the uterus or maternal diet. SLC2A1 is the main glucose transporter in the placenta and these results suggest that glucose transport within the placenta does not significantly differ from placentome to placentome and is unaffected by moderate maternal nutrient restriction. In addition, *SLC7A1* expression did not differ among placentomes or maternal diet suggesting that transport of amino acids was unaffected. Similarly, previous work in our lab has found no difference in expression of *SLC2A1* or *SLC7A1* in the ovine placenta at GD125 after ewes had been nutrient restricted to 50% of NRC requirements. Collectively, results suggest that analyses of gene expression for nutrient transporters within small, non-centralized placentomes represents expression of these transporters within the entirety of the population of placentomes.

The selection of *PAG1* and *CSH1* as candidate genes was primarily due to their known expression only within the cotyledonary portion of the placenta. We hoped to ascertain if cotyledonary development was similar in placentomes from the distal portion of the uterine horns compared to those more centralized to the fetus. Results indicate that expression of PAG1 and CSH1 did not differ based on location of placentomes within the uterus or by maternal diet. These results suggest that the development of cotyledonary tissue and expression of cotyledonary specific hormones is not different between small placentomes from the tips of the uterine horns and larger placentomes more central to the fetus. PAG1 is thought to play an immunological role in the placenta as it is most abundant during mid- and late-gestation, but decreases near the time for

parturition. Furthermore, PAG1 is thought to possess a luteotropic role, maintaining elevated progesterone for the maintenance of pregnancy. Expression of *PAG1* was unaffected by location of placentome within the uterus and maternal diet suggesting that immune function and maintenance of pregnancy was unaffected by those parameters. Furthermore, *CSH1* expression did not differ based on placentome location within the uterus or maternal diet. CSH1 is synthesized by binucleate cells and increases in concentration during mid and late gestation (Green et al., 2000; Hashizume et al., 2007).

Estrogen is a potent vasodilator and likely plays a role in enhancing uteroplacental blood flow in late gestation (Rosenfeld et al., 1996). Estrogen receptors are located in the caruncle while placental estrogens are mainly produced by the cotyledon (Evans and Wagner, 1981; Larsson et al., 1981; Gross and Williams, 1988; Hoedemaker et al., 1990; Hoffman and Schuler, 2002). With placental estrogen being a primary regulator of placental blood flow, differences in levels of expression of *ESR1* would potentially indicate variability in caruncular responsiveness to the vasodilatory actions of placental estrogens. Nonetheless, the expression of receptors for vasodilatory hormones in the small, non-centralized placentomes represents the expression levels of these receptors throughout the uterus. Similarly, the growth promoting hormone, *IGF1* and a regulator of its bioavailability, *IGFBP3* were unaffected by location of placentome or by maternal diet at GD270 suggesting that the glucose-insulin-insulin-like growth factor axis is uniformly expressed throughout the placenta.

Collectively, results of the present study indicate that expression of mRNAs of a number of critically important genes known to play a role in placental and/or fetal

development does not differ among locations within the uterus. In fact, variation among individual animals was highest, followed by variation due to diet, and variation was the least among placentome location. While, at first glance, these negative data may not appear to be of critical importance to our understanding of placental biology, the results of the present study are an essential first step in developing non-terminal sampling methodologies to investigate the relationship between environmental insults in utero and postnatal phenotype of the offspring. Our laboratory has recently developed a surgical technique in the sheep that allows for the removal of a single placentome without compromising pregnancy. This novel methodology allows for the retrospective assessment of placental gene expression based on an observed postnatal phenotype in the same animal. Importantly, results of the present study support the rationale that the removal of that single placentome would, in fact, be representative of the entire population of placentomes within the uterus. In the sheep, the placenta is capable of producing sufficient quantities of progesterone to maintain pregnancy after GD60. This is important, as it is expected that tactile manipulation of the uterus during the placentomectomy procedure would cause the release of prostaglandin F2 alpha. If luteolytic quantities of prostaglandins are released prior to the placental takeover of progesterone production the pregnancy may be at risk. The cow is more susceptible to pregnancy loss in response to prostaglandin F2 alpha release due to the fact that the bovine placenta does not produce sufficient quantities of progesterone to maintain pregnancy until much later in gestation. Therefore, the ability to sample a placentome from the contralateral uterine horn either by surgery or biopsy may greatly reduce the

risk of pregnancy loss while allowing for collection of placental tissues that fully represent the placental tissues in regions in closer proximity to the fetus and corpus luteum. Results of the present study support the continued efforts to develop methodologies to sample placentomes from the contralateral uterine horn as an additional tool for reproductive biologists to better understand the relationship between placental gene expression and development of programmed postnatal phenotypes.

CHAPTER IV

CONCLUSIONS

Proper placental development is vital for the survival and nourishment of the fetus. Inadequate placental development leads to inadequate nutrient and waste exchange to the fetus which can contribute to negative postnatal health and performance. Placental gene expression for factors vital for angiogenesis, nutrient transport, hormonal action, and cotyledonary specific factors is not affected by location of the placentome within the gravid bovine uterus. These findings suggest that representative placental gene expression can be measured by surgical removal of a placentome located in the contralateral uterine horn. This would decrease the risk of pregnancy loss due to manipulation of the uterus releasing prostaglandins in the ipsilateral uterine horn creating a more successful model to study nutrient restriction's effects on placental and fetal development. As ruminants with similar placental development, these results from studies of the bovine placenta should be applicable to sheep and goats; however, a similar study should be conducted using sheep to determine the efficacy of surgical placentome removal from the contralateral uterine horn for use in gene expression analysis.

Furthermore, placental gene expression for angiogenic factors, nutrient transporters, factors associated with hormone action, and cotyledonary specific factors is unaffected by mild maternal nutrient restriction at GD270. Future studies need to identify the effects, if any, of mild nutrient restriction on placental gene expression and

function during periods of placental development as the bovine placenta is fully developed and functional at GD270 and differences in gene expression have the potential to be identified during earlier stages of gestation. In addition, research can be done to identify if gene expression in placentomes is altered by more severe maternal nutrient restriction as is often used in sheep models of nutrient restriction (Zhu et al., 2004; Gilbert et al., 2005; Zhu et al., 2006; Heasman et al., 1998).

Maternal nutrient restriction contributes significantly to negative impacts on health, carcass quality, and reproductive characteristics in cattle. Determining the mechanisms by which these negative impacts are occurring will allow for mitigation of the negative impacts of fetal programming. In turn, producers of all phases of the beef industry will have the potential to produce more efficient and profitable cattle.

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